Chaperonin-Affected Folding of Globular Proteins

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Abstract. We studied the effect of GroEL on the kinetic refolding of α -lactalbumin by stopped-flow fluorescence techniques. We used wild-type GroEL and its ATPase-deficient mutant D398A, and studied the binding constants between GroEL and the molten globule folding intermediate at various concentrations of ADP and ATP. The results are compared with titration of GroEL with the nucleotides, ADP, ATP-analogs (ATP- γ S and AMP-PNP) and ATP, which have shown that both ADP and the ATP analogs are bound to GroEL in a non-cooperative manner but that ATP shows a cooperative effect. Similarly, the binding constant between GroEL and the folding intermediate decreased in a cooperative manner with an increase in ATP concentration although it showed non-cooperative decrease with respect to ADP concentration. It is shown that the allosteric control of GroEL by the nucleotides is responsible for the above behavior of GroEL and that the observed difference between the ATP- and ADP-induced transitions of GroEL is brought about by a small difference in an allosteric parameter (the ratio of the nucleotide affinities of GroEL in the high-affinity and the low-affinity states), i.e., 4.1 for ATP and 2.6 for ADP.

Key words: chaperonin, GroEL, molecular chaperone, protein folding

Although the protein folding observed experimentally *in vitro* often occurs spontaneously, the folding of a protein *in vivo* is not a spontaneous process but actively mediated by a number of molecular chaperones. Molecular chaperones recognize and transiently bind to folding intermediates of proteins, and prevent irreversible aggregation that is often irretrievable at such high concentrations of proteins as in cellular environment. Elucidation of molecular mechanism of molecular chaperone action is thus to understand the relationship between the uni-molecular folding reaction and much more complex phenomena within the biological cell.

Among various molecular chaperones, the best characterized physicochemically is the chaperonin GroEL from *Escherichia coli* [1, 2]. GroEL is a 14-mer protein of two rings, which are stacked back to back on each other, and each ring consists of seven 57-kDa subunits. Each subunit consists of three domains: an equatorial domain that contains a nucleotide binding site as well as most of the interaction sites between monomers and most of the contact regions between the heptameric rings; an apical domain that contains a binding site for a target protein and GroES; and an intermediate domain that connects the equatorial and apical domains (Figure 1). GroEL has a weak ATPase activity, and the full functional activity of GroEL requires the co-chaperonin GroES and ATP. GroES composed of

Figure 1. The structure of the *E. coli* chaperonin GroEL (PDB code: 1OEL). (a) A space-filling model of the GroEL particle (the top view (upper) and the side view (lower)). (b) A ribbon model of a subunit of GroEL.

seven 10 kDa subunits has a dome-shaped structure, and the GroEL/ES complex stabilized by binding with ATP or ADP forms a large central cavity of the complex. A target protein of GroEL is encapsulated within this cavity of the ATP-GroEL/ES complex and undergoes productive folding in this location [3].

In this article, we first briefly summarize our recent studies on (1) titration of GroEL by nucleotides (ADP and ATP analogs (ATP γ S and AMP-PNP)) by isothermal titration calorimetry (ITC) and by fluorescence spectroscopy of pyrene-labeled GroEL [4, 5], and (2) GroEL-affected refolding kinetics of α -lactalbumin (α LA) and staphylococcal nuclease (SNase) studied by tryptophan fluorescence spectroscopy [6–8]. Then, (3) we describe our most recent study on effects of ATP and ADP on the GroEL-affected refolding of α LA [9, 10]. GroEL alone has some chaperone activity for a number of target proteins in the presence of ATP [11–14], and as a first step for fully understanding the molecular mechanism of the complicated chaperonin function, we have successfully obtained quantitative description about the allosteric control of the GroEL target recognition induced by the nucleotide.

Figure 2. Initial velocities of ATP hydrolysis by GroEL at different concentrations of ATP. The reactions were carried out in standard buffer (pH 7.5) at 23 °C. The monomeric concentration of protein was 420 nM. The initial velocities of the ATP hydrolysis at ATP concentrations below 100 μ M (a) and at higher concentrations of ATP (b). From [5] with permission.

1. Titration of GroEL by ADP and ATP Analogs

GroEL undergoes allosteric transitions with respect to ATP, and the transitions seem to be important for the chaperonin function. Figure 2 shows the initial rate of ATP hydrolysis by GroEL as a function of ATP concentration. The rate first increases in a cooperative manner between 5 and 30 μ M ATP, then decreases above ~ 150 μ M ATP [5]. The first increase in the rate is represented by a MWC (Monod-Wyman-Changeux) allosteric model with a positive cooperativity within a ring, in which each subunit can assume two conformational states, T and R states (equation 1), while the second transition is represented by a KNF (Koshland-Némethy-Filmer) allosteric model with a negative cooperativity between the two rings (equation 2) [15–19]. Thus, the two allosteric transitions of GroEL are given by

where T denotes the T state in which all the seven subunits within a single ring is in the same T (tense) conformation, and R denotes the R state in which all the seven subunits is in the same R (relaxed) conformation. TT, TR, and RR denote the two-ring states of the whole GroEL particle, and it changes from TT to RR via TR with increasing ATP concentration.

To investigate the nucleotide-induced transitions of GroEL, we performed the ITC titration of wild-type GroEL and its single-ring mutant (SR1) and the fluorescence-detected titration of the pyrene-labeled forms of the two proteins with ADP and ATP analogs (ATP γ S and AMP-PNP) [4, 5]. Rather surprisingly, however, our results indicated that the binding of ADP and ATP analogs to either wild-type GroEL or SR1 was non-cooperative. Only ATP induces an apparently cooperative

Figure 3. Calorimetric titration of SR1 with ATP γ S at 5.6 °C analyzed by the non-cooperative and positively cooperative binding models. Experimental data points are those from the titration of ATP γ S to SR1. A solid line is the best fit of the data to the non-cooperative model. An increase of binding at the beginning of titration, which was expected if the positive cooperativity existed, was not observed. The best fit curve with the fixed n_H of 2 by the cooperative binding model is also given by a broken line. From [5] with permission.

conformational change in both the proteins. Furthermore, the fluorescence changes of pyrene-labeled GroEL indicated that GroEL (wild-type and SR1) has two kinds of nucleotide binding sites. The fluorescence titration results fit well with a model in which two kind of binding sites are both non-cooperative and independent of each other.

Figure 3 shows a typical example of the ITC titration of the protein (SR1 in this case) with ATP γ S [5]. A solid line shows the theoretical curve based on a non-cooperative binding model, in which the saturation fraction Θ of the nucleotide binding is given by

$$\Theta = \frac{K_s[S]}{1 + K_s[S]}, \quad (3)$$

where K_s represents the binding constant between the protein and the nucleotide, and $[S]$ is concentration of the nucleotide. The theoretical curve shows a good agreement with experimental data. A broken line in Figure 3 represents the theoretically best-fit curve based on a cooperative binding model, in which the Θ is given by

$$\Theta = \frac{K_{app}[S]^{n_H}}{1 + K_{app}[S]^{n_H}}, \quad (4)$$

Figure 4. Kinetic refolding curves of apo- α -LA (a) and SNase (b) monitored by stopped-flow fluorescence in the absence (curve 1) and the presence (curve 2) of GroEL. Background fluorescence changes due to the presence of GroEL is corrected in curve 2. (a) The refolding was initiated by a pH jump from pH 2.5, where apo- α -LA is in the molten globule state, to pH 7.0, where the protein is in the native state. The refolding rate constants in the absence and the presence of GroEL are 0.076 s^{-1} and 0.037 s^{-1} , respectively. (b) The refolding was initiated by a pH jump from pH 2.5, where SNase is in the acid-unfolded state, to pH 7.5, where the protein is in the native state (Tsurupa, G. and Kuwajima, K., unpublished data).

where K_{app} is the apparent binding constant, and n_H , the Hill coefficient representing the cooperativity of the transition, is kept constant ($n_H = 2$) for the broken line in Figure 3. The theoretical curve based on the cooperative model does not show reasonable agreement with the experimental data. When we took n_H as a fitting variable and performed the non-linear least squares fitting of the experimental data to equation 4, the n_H was less than 1.2 for all the ITC binding data between the protein (wild-type GroEL or SR1) and the nucleotide (ADP or ATP γ S). These results thus clearly demonstrate that there is no positive cooperativity in the nucleotide binding to GroEL.

If there is no cooperative behavior in the binding of ADP and the ATP analogs to GroEL, why is the ATP hydrolysis by GroEL cooperative (Figure 2)? A question may thus arise: Is the cooperative effect of ATP is caused by ATP binding or does it require ATP hydrolysis? A more recent study has shown that the ATP binding is indeed responsible for the cooperative effect and that the difference between ATP and the other nucleotides is interpreted in terms of a difference in an allosteric parameter between the nucleotides (see below) [10].

2. GroEL-Affected Kinetic Refolding of α LA and SNase

To elucidate quantitatively how strongly GroEL binds to a transient folding intermediate of a protein, we investigated the effect of GroEL on the refolding kinetics

of α LA and SNase [6–8]. GroEL retards the refolding kinetics of these proteins by interacting with a transient folding intermediate as evidenced by dependence of the retardation effect on GroEL concentration. Figure 4 shows kinetic refolding curves of apo- α -LA and SNase in the absence and presence of GroEL, studied by stopped-flow fluorescence measurements. GroEL retards the refolding of both proteins, but the effect is much more dramatic for SNase. This difference has been interpreted in terms of an electrostatic effect. The net charge at neutral pH is -7 and $+12$ for apo- α -LA and SNase, respectively, and the stronger retardation effect for SNase may be due to this charge difference. GroEL is highly negatively charged (-18 per monomer) at neutral pH, so that basic proteins that have positive net charge are more strongly bound to GroEL than acidic ones. GroEL binds to a target protein mainly by hydrophobic interactions, but the present result demonstrates that the electrostatic interaction is also a dominant factor determining the strength of the interaction between GroEL and its target protein.

The effects of GroEL on the refolding kinetics of the two proteins (α LA and SNase) are, however, both represented by scheme 1 [8].

Scheme 1.

In this scheme, GroEL (G) binds the refolding intermediate (the molten globule state) (A) and retards the apparent refolding rate k_{app} of the protein. For apo- α -LA, the binding equilibrium between G and A occurs very rapidly compared with the free refolding (process 1), and the refolding in the presence of GroEL occurs exclusively through process 2 with the k_{app} given by

$$k_{app} = \frac{k_f}{1 + K_b[G]}, \quad (5)$$

where k_f and K_b are the rate constant for the free refolding and the binding constant between G and A; K_b is the ratio of the rate constants for binding and dissociation ($K_b = k_b/k_d$). For SNase, the binding of GroEL to the intermediate is so strong that the intermediate refolds in the GroEL-bound state (process 2) with a rate constant k_g . The refolding and binding parameters under the influence of GroEL have been evaluated quantitatively on the basis of scheme 1. The K_b values for apo- α -LA and SNase are $7 \times 10^5 \text{ M}^{-1}$ and larger than 10^9 M^{-1} , respectively.

The simplicity of the refolding kinetics of apo- α LA allowed us to evaluate the K_b between GroEL and the α LA folding intermediate quantitatively. Thus, we

Figure 5. The binding constants K_b between GroEL (light bars) (or D398A (dark bars)) and the refolding intermediate of α LA in the presence and the absence of nucleotides. The K_b values were estimated by the fitting of the experimental data to the theoretical curve with scheme 1. From [10] with permission.

also investigated the GroEL-affected kinetic refolding of apo- α LA in the absence and presence of the nucleotide (ADP, AMP-PNP, or ATP at 1 mM) and the co-chaperonin GroES. When GroES was absent, ATP remarkably reduced the affinity of GroEL for the intermediate of α LA (Figure 5). When GroES was present, not only ATP but also ADP and AMP-PNP were effective in reduction of the affinity for the intermediate [9].

3. Effects of ATP and ADP on the GroEL-Affected Refolding of α LA

3.1. THE CHANGE IN THE GROEL AFFINITY FOR THE TARGET PROTEIN CAUSED BY THE ALLOSTERIC TRANSITION

The reduction of the affinity of GroEL for the α LA intermediate by ATP may be consistent with the MWC model in which the allosteric transition from the T to the R state by ATP decreases the affinity of GroEL for the target protein. However, there was a question: Is it ATP binding or ATP hydrolysis that decreases the affinity of GroEL? Because we observed the non-cooperative binding of the nucleotides, including the ATP analogs, this question seemed to be serious. For the purpose of resolving the question, we thus employed both wild-type GroEL and its ATPase-

deficient mutant D398A to investigate the GroEL-affected refolding kinetics of α LA at various concentrations of ATP and ADP. The replacement of the wild-type Asp398 by alanine in D398A diminishes the ATPase activity of GroEL but does not remarkably change the equilibrium binding capacity to ATP [20], so that we can separately investigate the effects of ATP binding and ATP hydrolysis using both wild-type GroEL and D398A. In this study, we also addressed a question: If the reduction of the GroEL affinity is due to the nucleotide binding, how does the allosteric control of GroEL by the nucleotide affect the GroEL-mediated kinetic refolding of a target protein? The availability of the quantitative data of the GroEL-affected kinetic refolding for apo- α LA has made this protein particularly useful as a model target protein for investigating the above issues.

Figure 5 shows the binding constants of wild-type GroEL and D398A with the α LA folding intermediate in the presence and absence of the nucleotides (ADP and ATP) [10]. The binding constants were obtained by numerical simulation analysis of the kinetic refolding curves of apo- α LA in the absence and presence of GroEL (or D398A) with equation 5. Both ADP and ATP remarkably reduce the affinity of GroEL for the intermediate, and this effect is observed not only in wild-type GroEL but also in D398A.

To investigate more rigorously the reduction of the affinity of D398A by ATP, the α LA refolding was started at different periods of time (τ) after addition of ATP (scheme 2), and the K_b values were calculated.

Scheme 2.

The ATPase reaction of wild-type GroEL is known to cycle in every 15 seconds, and this is faster than the time required for the α LA refolding (about 30 seconds; Figure 4). However, the ATPase cycle of D398A is much slower than that of wild-type GroEL, and it takes more than 6 minutes to complete one ATPase cycle under a steady-state condition (data not shown). Therefore, if GroEL reduced its affinity for the target protein in cooperation with the ATPase cycle, the time-dependent change in the apparent binding constant K_b of D398A would be observed with increasing the incubation time with ATP. Figure 6 shows the K_b of D398A as a function of the incubation time. The results clearly show that the reduction of the K_b of D398A occurs within the shortest incubation time (15 seconds) employed, which is much shorter than the time required for completing one ATPase cycle of D398A. The reduction of the K_b values of D398A and probably also wild-type GroEL for the target protein is thus not coupled with the steady-state ATPase cycle. These results

Figure 6. The apparent binding constant K_b between D398A and the α LA refolding intermediate as a function of the pre-incubation time (τ) of ATP in the D398A solution before starting the α LA refolding. The final concentrations of D398A, α LA and ATP were $1.0 \mu\text{M}$, $1.0 \mu\text{M}$, and $200 \mu\text{M}$, respectively. The cross indicates the K_b between D398A and the α LA refolding intermediate in the absence of ATP. From [10] with permission.

thus suggest that the ATP binding itself induces a conformational change of GroEL from the high affinity (T) to the low affinity state (R) for the target protein; these are the low-affinity and high-affinity states, respectively, for ATP. We have also studied the ATP hydrolysis by GroEL, and the absence of the initial burst of the ATP hydrolysis has further shown that the T to R transition is not coupled with the ATP hydrolysis but brought about by the ATP binding (Inobe et al., unpublished data).

3.2. ANALYSIS OF THE ATP-CONCENTRATION DEPENDENCE OF k_b ON THE BASIS OF THE MWC MODEL

To investigate the T to R conformational transition more in detail, we measured the GroEL- (or D398A-) affected refolding kinetics of apo- α LA at various concentrations of ATP, and analyzed the ATP-concentration dependence of K_b (Figure 7) [10]. We can see from Figure 7 that there is a cooperative transition for both wild-type GroEL and D398A from the high-affinity to the low-affinity state for the target protein as measured by the dependence of K_b on ATP concentration and

Figure 7. The binding constants K_b between the α LA refolding intermediate and GroEL (circles) (or D398A (triangles)) at various concentrations of ATP. The K_b between GroEL (or D398A) and the α LA refolding intermediate was obtained by the fitting of the experimental data to the theoretical refolding curve with scheme 1. The lines shown in the figure are obtained by the fitting of the K_b values to equation 9 that is based on scheme 3 (the MWC model). Error bars shown in the figure are the standard deviations from three independent measurements for each data point. The inset shows a magnified plot along x-axis. From [10] with permission.

that this transition occurs at similar or slightly higher concentrations of ATP than the transition measured by the initial rate of ATP hydrolysis (Figure 2). The shift of the transition to a higher ATP concentration may be due to stabilization of the T state by the binding of α LA to GroEL.

The results of Figure 7 were, thus, analyzed with scheme 3 that is based on the MWC model. In this scheme, all the seven subunits in the single ring of GroEL are assumed to be in the equilibrium between the two states, T and R. GroEL is predominantly in the T state in the absence of the nucleotide, such that

$$L = \frac{[T]}{[R]} \gg 1. \quad (6)$$

The partition function Z of the system is given by

Scheme 3.

$$\begin{aligned}
 Z &= Z_T + Z_R \\
 Z_T &= [T](1 + K_S^T[S])^7 \\
 Z_R &= [R](1 + K_S^R[S])^7,
 \end{aligned} \tag{7}$$

where K_S^T and K_S^R are the binding constants between the nucleotide and GroEL in the T and R states, respectively. The exponent of 7 in equation 7 reflects the presence of 7 subunits in a single ring of GroEL. The fractional saturation of the nucleotide-binding sites in GroEL Θ is thus given by

$$\Theta = \frac{1}{7} \frac{\partial \ln Z}{\partial \ln [S]} \tag{8}$$

Table I. Best-fit parameters obtained by MWC model (scheme 3) *: fixed

		$K_b^T (10^6 \text{ M}^{-1})$	$K_S^R (10^3 \text{ M}^{-1})$	β	γ
Wild-type	ATP	3.47*	137 ± 9	0.152*	4.06 ± 0.14
	ADP	3.47*	44 ± 4	0.152*	2.58 ± 0.02
D398A	ATP	3.20*	179 ± 7	0.188*	4.17 ± 0.06
	ADP	3.20*	22 ± 2	0.188*	3.21 ± 0.07

$$= \frac{K_S^R [S] (1 + K_S^R [S])^6}{L(1 + K_S^T [S]/\gamma)^7 + (1 + K_S^R [S])^7}.$$

The apparent binding constant K_b between GroEL and the refolding intermediate of α LA is thus obtained by

$$K_b([S]) = \frac{K_b^T Z_T + K_b^R Z_R}{Z} \quad (9)$$

$$= K_b^T \frac{L(1 + K_S^R [S]/\gamma)^7 + \beta(1 + K_S^R [S])^7}{L(1 + K_S^R [S]/\gamma)^7 + (1 + K_S^R [S])^7},$$

where K_b^T and K_b^R are the K_b values in the T and R states, respectively, and

$$L = \frac{[T]}{[R]}$$

$$\beta = \frac{K_b^R}{K_b^T} \quad (10)$$

$$\gamma = \frac{K_S^R}{K_S^T}.$$

The L value, the ratio between the GroEL concentrations in the high affinity and the low affinity state in the absence of nucleotide, was fixed to 500, which derived from Yifrach and Horovitz (1995). The β value was fixed to the value of $(K_b \text{ at } 1.0 \text{ mM ATP})/(K_b \text{ in the absence of ATP})$ that was obtained experimentally, because most of GroEL were in the T state in the absence of ATP and in the R state at 1 mM ATP.

To obtain the best-fit parameters values of scheme 3 from the experimental data, we performed the non-linear least-squares calculations of the data of Figure 7 with the Levenberg-Marquardt algorithm to minimize the sum of squares of residuals between the experimental and theoretical values of K_b . The parameter (K_b^T , K_S^R , β and γ) values obtained are summarized in Table I. The theoretical curves drawn with these parameter values are also shown in Figure 7. The theoretical curves well represent the experimental data of the dependence of K_b on ATP concentration.

Figure 8. The apparent binding constants K_b between the α LA refolding intermediate and wild-type GroEL (circles) and between the α LA intermediate and D398A (triangles) at various concentrations of ADP. The final concentrations of wild-type GroEL (or D398A) and α LA were both equal to 1.0 μ M. The K_b values shown in the figures were obtained by the fitting of the refolding curves to the theoretical curves with scheme 1. The lines were obtained by the fitting of the K_b values to equation 9, which were derived from scheme 3 (the MWC model). From [10] with permission.

3.3. ANALYSIS OF THE ADP-CONCENTRATION DEPENDENCE OF k_b ON THE BASIS OF THE MWC MODEL

We next investigated the interaction between wild-type GroEL (or D398A) and the refolding intermediate of α LA at various concentrations of ADP (Figure 8) [10]. ADP also decreased the apparent binding constant K_b , but the extent was smaller than that observed in ATP (Figure 7). There was no cooperative decrease in K_b with respect to ADP concentration, and this behavior is clearly different from that observed in ATP and is consistent with our previous observation that ADP is bound by GroEL in a non-cooperative manner [4, 5]. The K_b of D398A was, however, significantly smaller than that of wild-type GroEL over the whole range of ADP concentrations (Figure 8, triangles), and the difference between wild-type GroEL and D398A increased with ADP concentration.

We analyzed the ADP-concentration dependence of K_b on the basis of the MWC model (scheme 3). Because the parameter β was independent of the ligand species in the MWC model, the β value fixed to the value obtained from the transition

induced by ATP was used for the analysis of the transition induced by ADP as well. The parameter values obtained by the least-squares fitting of the data of Figure 8 are also listed in Table I, and the theoretical fitting curves with these parameter values well represent the experimentally observed dependence of K_b on ADP concentration (Figure 8).

3.4. WHY IS THE TRANSITION OF GROEL BY ADP APPARENTLY NON-COOPERATIVE?

The above results show that both the ATP- and ADP-induced changes in K_b are well represented by the allosteric model (scheme 3) although the apparently the cooperativity of the transition is quite different between ATP and ADP. To address a question of why the transition of GroEL is not cooperative by ADP in spite of the cooperative transition induced by ATP, we should describe the T to R transition directly using the analyses of the refolding curves of α LA in the presence of both GroEL and the nucleotide [10]. At an intermediate concentration of the nucleotide, both the T and R states of GroEL are populated significantly and interact with the target protein with the binding constants of K_b^T and K_b^R , respectively. We thus analyzed the refolding reactions of α LA in the presence of both GroEL and the nucleotide with scheme 4.

Scheme 4.

The K_b^T and K_b^R values were fixed to the experimentally observed K_b values in the T state in the absence of the nucleotide and in the R state at a saturating concentration of ATP, respectively. The fraction (α) of GroEL in the R state given by

$$\alpha = \frac{[G_R]}{[G_T] + [G_R]} \quad (11)$$

is thus the only fitting parameter for the analysis of the GroEL-affected refolding curves at different concentrations of the nucleotide. Here, $[G_T]$ and $[G_R]$ represent the concentrations of GroEL in the T and the R states, respectively, under a given condition; it should be noted that these are different from $[T]$ and $[R]$ (equations 6–10) that are the concentrations in the absence of any ligand.

Figure 9 shows the transition curves of wild-type GroEL from the T to R state induced by ATP and ADP. Almost all GroEL molecules were in the R state above 200 μ M ATP. However, ADP did not convert GroEL fully to the R state even at

Figure 9. The transition of GroEL from the T to the R state induced by ATP (circles) and ADP (triangles). The α values were obtained by the fitting of the refolding curve of α LA with scheme 4. The lines in the figure were obtained by the Hill analysis. From [10] with permission.

1.5 mM ADP, and this was the reason why the binding of ADP to GroEL was apparently non-cooperative. We also performed the Hill analysis of these data and obtained the Hill coefficients by equation 4. The Hill coefficients of wild-type GroEL for the transitions induced by ATP and ADP were $2.1 (\pm 0.2)$ and $0.9 (\pm 0.2)$, respectively. The transition midpoints of the two transitions were $29.8 (\pm 0.9) \mu\text{M}$ and $270 (\pm 130) \mu\text{M}$, respectively.

4. Conclusions

1. The present results provide a clear case that the ATP-induced allosteric transition of GroEL regulates the affinity for the transient folding intermediate of a target protein, and we have successfully obtained the quantitative estimates of the allosteric parameters associated with this regulation.
2. For the D398A mutant, the reduction of the affinity was found to occur much faster (within 15 seconds) than the steady-state ATP hydrolysis of this mutant (more than 10 minutes). This clearly indicates that the steady-state ATP hy-

drolysis is not required for the reduction of the affinity of GroEL for the target protein.

3. The transition midpoint of the ATP-induced transition of GroEL was about 30 μ M, which was very similar to that evaluated from other structural studies. This indicates that the reduction of the affinity of GroEL for the target protein is due to the structural change of GroEL induced by ATP.
4. Although ATP binding to GroEL induces the allosteric transition of GroEL, ADP binding to GroEL leads to only a non-cooperative change in the affinity for the target protein. The analysis based on the MWC model suggests that the γ value is a little smaller for ADP than for ATP and results in the apparently non-cooperative change by ADP.

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